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Aquatic Plant Control Research Program

# Assessment of Fungal Pathogens as Biocontrol Agents of *Myriophyllum spicatum*

by J. L. Harvey, Harry C. Evans, International Institute of Biological Control

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# Assessment of Fungal Pathogens as Biocontrol Agents of *Myriophyllum spicatum*

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Final report

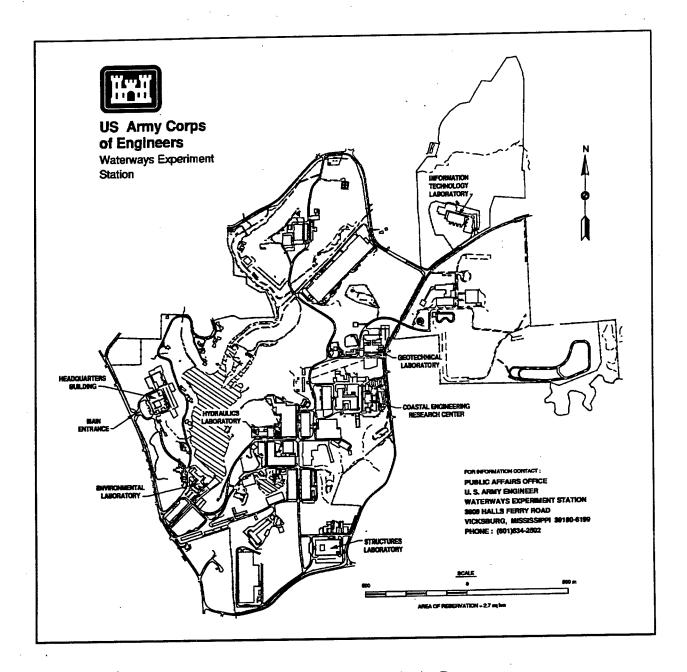
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## **Preface**

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit 32863. The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96X3122, Construction General. The APCRP is managed under the Center for Aquatic Plant Research and Technology (CAPRT), Dr. John W. Barko, Director. Mr. Robert C. Gunkel was Assistant Director for the CAPRT. Program Monitor during this study was Ms. Denise White, HQUSACE.

The Principal Investigator for the study was Dr. Harry C. Evans, International Institute of Biological Control, Silwood Park, Ascot, United Kingdom. He was assisted in the research by postdoctoral candidate, Dr. J. L. Harvey. The study was conducted and the report prepared by Drs. Evans and Harvey. The research coordinator at WES was Dr. Judy F. Shearer.

This investigation was performed under the general supervision of Dr. John W. Keeley, Director, EL; Dr. Conrad J. Kirby, Chief, Ecological Research Division (ERD), EL; and Dr. Alfred F. Cofrancesco, Jr., Chief, Aquatic Ecology Branch, ERD.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

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## 1 Introduction

Myriophyllum spicatum L. (or Eurasian watermilfoil) is a member of the Haloragaceae family. It is a submersed aquatic plant that grows in a wide range of environmental conditions, in both fresh and brackish water. In weedy situations, it is fast growing, forming dense mats of foliage that interfere with the normal usage of water courses. Reproduction is by fragmentation of stems and the development of overwintering buds; seed formation also occurs but may play little part in the spread of the weed.

Myriophyllum spicatum is widely distributed throughout the United Kingdom, with records from Cornwall through to the Outer Hebrides, and occurs in most European countries from Scandinavia in the North to Sicily in the South (Kew Herbarium records). It also occurs in most of Asia as well as in East Africa (Harley and Forno 1990). Although locally common throughout the natural range, it is rarely dominant and has never been reported as a weed problem. It is most frequently found in the U.K. in still water, especially in lime-rich areas. Other Myriophyllum spp. (i.e., M. alterniflorum and M. proserpinacoides) share its habitat, while M. verticillatum grows in faster flowing water.

Myriophyllum spicatum has been a problem in the United States since the 1930s (Harley and Forno 1990). In the 1950s and 1960s, it became a serious ecological and economical weed in larger bodies of water in North America. As an ecological problem, M. spicatum can greatly reduce the numbers of naturally occurring aquatic plant species, with records of a fall in species number from 20 to 9 in a 2-year period, with M. spicatum coverage increasing from 2 percent to 20 to 45 percent over the same period (Madsen et al. 1990).

Attempts to control *M. spicatum* have involved both mechanical and chemical methods. Mechanical clearance can be cheaper than chemical alternatives, but needs to be carried out at least twice during the summer to produce a reasonable reduction in plant biomass. Herbicide applications have been successful; both underwater application made by boat and aerial application can give good control. However, because of environmental concerns, application of chemical herbicides needs careful consideration. Due to the dilution from a body of water, large amounts of herbicide need to be applied; if control is not sufficient, reinfestation can be rapid. In addition, the chemical has

to be specific and persistent enough to control the weed with no residual activity.

Many of the early investigations into biological control agents for *M. spicatum* concentrated on insects. Species on other *Myriophyllum* spp. from within the United States have been identified as possible control agents. A pyralid moth, *Acentria nivea*, found in stands of *Myriophyllum exalbescens* in the St. Lawrence River caused leaf loss and girdling of stems (Batra 1977). Surveys predominantly for insect agents have also been carried out in Pakistan, Bangladesh, and much of Eastern Europe and Asia (Commonwealth Institute of Biological Control (CIBC) 1970; Harley and Forno 1990). However, many of the insects found proved to be nonspecific to the target weed and hence of limited use as biological control agents.

Use of pathogens has long been regarded as a good potential method of biological control for *M. spicatum* (Freeman and Charudattan 1980). Work has been undertaken on isolating and assessing fungal pathogens from within the United States: *Acremonium curvulum* and *Fusarium sporotrichoides* were tested at Wisconsin, but though capable of causing lesions, both failed to control the weed in large-scale tests (Andrews and Hecht 1981; Andrews, Hecht, and Bashirian 1982; Charudattan 1990).

A fungal pathogen, Colletotrichum gloeosporioides, found on M. spicatium in Wisconsin, has been evaluated as a mycoherbicide, in combination with three possible chemical herbicides at down to 10 percent of their recommended concentration (Sorsa, Nordheim, and Andrews 1988). Mycoleptodiscus terrestris, from the southern States, has also been tested against M. spicatum and a series of aquatic plants and terrestrial crop plants and has been shown to be virulent and reasonably specific (Verma and Charudattan 1993). Endophytic fungi have been reported in the literature on Myriophyllum sp. in both Europe and the United States (Sparrow 1974; Luther 1979) and appear to be damaging.

Myriophyllum spicatum constitutes part of the background or natural aquatic flora throughout most of Europe and rarely reaches weed status. However, some of the ecosystems (in Central Western Europe) have recently been invaded by the North American exotic species Myriophyllum heterophyllum (Spangehl and Scharrenberg 1986). Domination by the latter species would indicate that a different spectrum of natural enemies occurs in Europe and that a search for a fungal biological control agent for M. spicatum within Europe would be beneficial.

# 2 Material and Methods

#### Surveys

From plant records (Kew Herbarium, National Water Boards and the Terrestrial Ecological Surveys), sites of *M. spicatum* were selected to give a range of locations and environmental conditions. Sites were sampled over a 2-year period (1994-1995) during the growing season (May-October). Both *M. spicatum* and other *Myriophyllum* species were collected, and samples of water and soil were also taken in some cases. Samples were taken back to the weed pathology laboratories of the International Institute of Biological Control (IIBC), Silwood Park, Ascot Berks (U.K.)

#### Isolation

Isolation from diseased tissues of *M. spicatum* collected during the surveys was carried out following standard procedures; plants were washed under running tap water for 2 hr and rinsed in sterile distilled water before being placed on tap water agar (TWA). Samples of soil and water were also plated onto media selective for *Fusarium* (Komada 1975), and specific baits were employed for Oomycetes and aquatic fungi. Cultures were forwarded to the International Mycological Institute (IMI), Egham, Surrey (U.K.), for identification.

### Screening

Isolates of species that are commonly pathogenic to plants and those species that were isolated constantly from several sites were screened against M. spicatum.

Sections of plants (with two nodes) were cut, weighed (after excess surface water was removed), and placed in 100 ml of sterile distilled water in a jar. These were inoculated with either two 9-mm agar plugs or a  $10^4$  or  $10^6$  spores per milliliter suspension (dependent upon sporulation of the isolate) and kept at a constant 25 °C with 12 hr light. Two uninoculated controls were

included. After 3 weeks, plants were visually assessed for any indication of infection. After a further 2 weeks, samples were again visually assessed, reweighed (after excess surface water was removed), and plated onto TWA with antibiotics for identification and proof of pathogenicity (Koch's postulates). Comparison of initial and final weights was used to give an indication of inhibitory effect in the absence of physical signs of infection (it was noted during field collecting that plants generally show few lesions or other signs of infection).

# 3 Results and Discussion

#### Surveys

Over the two seasons of the project, surveys have been carried out at nearly 200 sites in 12 European countries, covering most of England, Wales, and Scotland, eastern France, northern Italy, northern Spain, northern Switzerland, southern Germany, central Austria, central Ireland, Portugal, and Slovenia (Appendix A). Sites from which *M. spicatum* was collected have varied in character from ponds and drainage ditches to large lakes, rivers, and canals. Plants were found in both still and fast-flowing water, and at depths from 5-8 cm to 4-5 m (in the clear waters of some of the southern European lakes). Though normally found in water of a neutral to alkaline pH, in a few sites in Scotland, *M. spicatum* was found in water that, due to surrounding peat, was mildly acidic. As the acidity increased, *M. spicatum* was replaced by *M. alterniflorium*.

Growth characteristics of the plants often varied, depending upon site features. In fast-flowing, shallow rivers, plants had noticeable red stems that trailed up to 1 m downstream and rooted at several points. In slower moving rivers and canals, plants had more branched stems, larger leaves (up to 3 cm in the Royal Canal, Ireland), and more surface detritus. In lakes, the major change in character was dependent upon the depth at which the plant was growing. Along the shallow edges of lakes, stems could be only a few centimeters long, increasing to several meters in deeper water. Plants grew deeper in the clearer and warmer southern European lakes compared with the more cloudy colder northern lakes in England and Scotland. When returned to the standard laboratory conditions, all plant samples grew in similar fashion, indicating that these are ecotypes rather than biotypes.

#### **Isolations**

From the plant material (*M. spicatum* and related species), water, and soil samples collected, over 400 isolates (from normally pathogenic genera) have been isolated, comprising 56 identified species in 39 genera (Appendix B). There was no correlation between the species isolated and the collection site, either environmentally or geographically. The majority of isolates are

common colonizers of plant tissues and genera. Fusarium and Acremonium have been routinely isolated from all types of locations. Significantly, Gliocladium roseum has only been isolated from lakes and ponds, not from rivers. A few isolates are specific aquatic fungi; e.g., Cylindrocarpon aquaticum and Nectria lugdunensis from the Crinnean Canal in Scotland. Several isolates have been unusual records, such as the two Embellisia sp. isolated from Texel in Holland and Slapton Ley in England, which had only previously been recorded from desert soils in Wyoming. Sclerotium hydrophilum, isolated from Afrilzer See in Austria, has previously been recorded on M. spicatum in Yugoslavia (IMI Culture collection).

### **Screening**

In total, 291 isolates have been tested; of these, 15 have shown some degree of pathogenicity or control, causing a reduction in growth (assessed by weight) and in more severe cases, loss of leaves, necrosis, or death (Appendix C). The majority of isolates damage the older tissue of the plant and have only a minimum effect on the newer growth. Of the isolates giving some degree of control, 12 of these were reisolated from plant tissues.

Of these 12 isolates that satisfy Koch's postulates, two are still unidentified Hyphomycetes (Mir 49a and Mir 80c) and two Coelomycetes (Mir 35 and Mir 36). Identification has been hampered by their very low and sporadic sporulation, though this does not appear to hinder either infection or reisolation from plant tissues.

Three of the isolates showing some degree of control are similar to those already screened in the United States (Andrews and Hecht 1981; Andrews, Hecht, and Bashirian 1982; Charudattan 1990; Verma and Charudattan 1993). Acremonium sp. (Mir 68c) has been screened twice giving good results and was reisolated both times. Results of reisolation of Fusarium sporotrichoides (MIR 96b) are still pending, but the isolate has been able to cause the death of inoculated plant sections. The native American isolates screened, Acremonium curvulum and Fusarium sporotrichoides (Andrews and Hecht 1981; Andrews, Hecht, and Bashirian 1982; Charudattan 1990), were successful in small-scale tests, but failed to control the weed in large trials. Though this may be the case with the European isolates, their closer evolution with the plants should allow for more consistent results. An isolate of Colletotrichum gloeosporioides (teleomorph: Glomerella cingulata) has been tested as a mycoherbicide in the United States (Sorsa, Nordheim, and Andrews 1988) while the European strain (Mir 51), though not reisolated from plant tissue, has been screened twice and reduced growth rate in both tests. Significantly, Mycoleptodiscus terrestris, which has been isolated in both the United States and China and shown to be virulent and reasonably specific to M. spicatum (Verma and Charudattan 1993), was not found during any of the European surveys.

Several of the isolates that have shown a degree of control (Cylindrocarpon destructans, Fusarium solani, Coniothyrium fuckelii, Geotrichum candidum, and Gliocladium roseum) are generally not regarded as pathogenic or specific. Their ability to infect M. spicatum was probably opportunistic, aided by the small plant sections used in the screen, and may not be repeatable with whole plants.

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# **Appendix A Locations of Collection Sites**

Table A1		
Code	Date	Site
MIR 1-9	9/4/93	Slapton Ley, Slapton, Devon, England
MIR 10-12	9/5/93	Cherrybrook, High Dartmoor, Devon, England
MIR 13	10/12/93	Streets Heath Pond, Chobham, Surrey, England
MIR 14	10/12/93	Wood Street Pond, Guilford, Surrey, England
MIR 15	posted	Southern Prague Lakes, Czech Republic
MIR 16	11/3/93	Wicken Fen Canal, Cambridgeshire, England
MIR 17	11/6/93	South Ferring Pond, Worthing, Sussex, England
MIR 18	8/28/93	Angermünde, Germany
MIR 19	9/1/93	Großer Buckowsee, Eberswalde, Germany
MIR 20	9/3/93	Oder-Havel-Kanal, Eberswalde, Germany
MIR 21	7/27/93	Marzurskie, Poland
MIR 22	posted	Heider See, Bonn, Germany
MIR 23	1/2/94	De Koog Pond, Texel Island, Netherlands
MIR 24	5/12/94	Wood Street Pond, Guilford, Surrey, England
MIR 25	5/25/94	Pett Pond, Winchelsea, Sussex, England
MIR 26	5/25/94	Drainage Ditch, Winchelsea, Sussex, England
MIR 27	5/25/94	St. Michaels on Wyre, Fleetwood, Lancashire, England
MIR 28-31	6/9/94	Roe Ponds, Hardwick Hall, Derbyshire, England
MIR 32	6/9/94	Fedder Pond, Chatsworth Palace, Derbyshire, England
MIR 33	6/20/94	Finger Pond, Priory Park, Bedfordshire, England
MIR 34	6/20/94	Harold and Odell Lake, Harold, Bedfordshire, England
		(Sheet 1 of 7)

Table A1 (Continued)			
Code	Date	Site	
MIR 35	6/20/94	River Great Ouze, Harold and Odell Country Park, Bedford- shire, England	
MIR 36	6/21/94	Soham Lode, Soham, Cambridgeshire, England	
MIR 37	6/28/94	River Axe, Axminster, Devon, England	
MIR 38	6/28/94	River Axe, Colyford, Devon, England	
MIR 39	6/28/94	Exeter Canal, Countess Weir, Devon, England	
MIR 40	6/28/94	Exeter Canal, Topsham Lock, Exminster Marshes, Devon, England	
MIR 41	4/29/94	Slapton Ley, Slapton, Devon, England	
MIR 42	4/29/94	River Frome, Moreton, Dorset, England	
MIR 43	4/29/94	River Piddle, Wool Bridge, Dorset, England	
MIR 44	7/5/94	Pit 16, Cotswold Water Park, Gloucestershire, England	
MIR 45	7/5/94	Lake 31, Keynes Country Park, Gloucestershire, England	
MIR 46	7/5/94	Lake 32, Keynes Country Park, Gloucestershire, England	
MIR 47	7/5/94	Lake 56, Neighbridge Country Park, Gloucestershire, England	
MIR 48	7/5/94	Lake Below 56, Neighbridge Country Park, Gloucestershire, England	
MIR 49	7/6/94	River Hart, Bramshill, Hampshire, England	
MIR 50	7/6/94	Whitewater River, North Warnborough, Hampshire, England	
MIR 51	7/6/94	Basingstoke Canal, Broad Oak Bridge, Hampshire, England	
MIR 52	7/6/94	Basingstoke Canal, Dogmersfield, Hampshire, England	
MIR 53	7/16/94	River Great Ouze, Clapham, Bedfordshire, England	
MIR 54	7/19/94	River Wylye, Great Wishford, Wiltshire, England	
MIR 55	7/19/94	River Nadder, Compton Chamberlayne, Wiltshire, England	
MIR 56	7/19/94	River Stour, Childe Okeford, Wiltshire, England	
MIR 57	7/19/94	River Stour, Dudsbury Golf Course, West Parley, Dorset, England	
MIR 58	7/20/94	River Avon, Kingston, Dorset, England	
MIR 59	7/20/94	Dockens Water, Rockford, Hampshire, England	
MIR 60	7/20/94	Funtley Lake, Funtley, Hampshire, England	
MIR 61	7/28/94	Burton Mere Trout Pond, Burton, South Wirral, Cheshire, England	
MIR 62	8/4/94	Kenfig Pond, South Glamorgan, Wales	
MIR 63	8/4/94	Llangorse Lake, Powys, Wales	
		(Sheet 2 of 7)	

Table A1 (Continued)			
Code	Date	Site	
MIR 64	8/4/94	Llan Bwch-llyn Lake, Powys, Wales	
MIR 65	8/5/94	Broxwood Court Pond, Broxwood, Hereford and Worcestershire, England	
MIR 66	8/5/94	River Arrow, Ivington, Hereford and Worcestershire, England	
MIR 67	8/17/94	Needham Market Fishing Lake, Needham Market, Suffolk, England	
MIR 68	8/18/94	Chantry Point Ditch, Orford, Suffolk, England	
MIR 69	9/2/94	Llangorse Lake, Powys, Wales	
MIR 70	9/5/94	River Eamont, Ullswater, Cumbria, England	
MIR 71	9/5/94	Derwent Water, North of Derwent Isle, Cumbria, England	
MIR 72	9/5/94	Derwent Water, East of Lords Isle, Cumbria, England	
MIR 73	9/6/94	Buttermere, Cumbria, England	
MIR 74	9/6/94	Crummock Water, Cumbria, England	
MIR 75	9/6/94	Ennerdale Water, Cumbria, England	
MIR 76	9/6/94	Loweswater, Cumbria, England	
MIR 77	9/7/94	River Irt, Wastwater, Cumbria, England	
MIR 78	9/7/94	Nether Beck, Wastwater, Cumbria, England	
MIR 79	9/7/94	Conniston Water, Cumbria, England	
MIR 80	9/7/94	Grasmere, Cumbria, England	
MIR 81	9/7/94	Rydal Water, Cumbria, England	
MIR 82-83	9/17/94	Lough Ree, River Shannon, Barley Harbour, Ireland	
MIR 84	9/17/94	Royal Canal, Ballynacargy, Ireland	
MIR 85	9/18/94	The Grand Canal, Edenderry, Ireland	
MIR 86	9/18/94	Lough Derravaragh, Castle Pollard, Ireland	
MIR 87	9/18/94	The Grand Canal, Rathangas, Ireland	
MIR 88	9/18/94	River Slate, Rathangas, Ireland	
MIR 89	9/18/94	River Liffey, Clane, Ireland	
MIR 90	10/3/94	Lake Geneva, Nyon, Switzerland	
MIR 91	10/4/94	Lake Neuchâtel, Colombie, Switzerland	
MIR 92	10/4/94	Etang De Maissausis, La Chapelle sous Chaux, France	
MIR 93	10/5/94	Lac de Longmer, Langmer, Gerardmer, France	
MIR 94	10/5/94	Stream north of Schaenau, Rhinau, France	
MIR 95	10/5/94	River Rhine, Rhinau, France	
		(Sheet 3 of 7)	

Table A1 (Continued)			
Code	Date	Site	
MIR 96	10/5/94	Canal du Rhône au Rhin, Neunkirch, France	
MIR 97	10/5/94	Etang de Stock, Diane-et-Kerpick, Gorraie, France	
MIR 98	10/6/94	River Moselle, Trieir, Germany	
MIR 99	10/7/94	Feilinger See, west of Koblenz, Germany	
MIR 100	4/29/95	Loch Ness Centre Pond, Drummandrochit, Highland Region, Scotland	
MIR 101	4/30/95	Pond off River Moristin, Glen Moriston, Highland Region, Scotland	
MIR 102	4/30/95	River Moristin, Glen Moriston, Highland Region, Scotland	
MIR 103	4/30/95	River Schiel, near Loch Duich, Highland Region, Scotland	
MIR 104	5/1/95	Tarn off Road, Kilmalaug, Isle of Skye, Highland Region, Scotland	
MIR 105	5/1/95	Tarn at Staffin, north Skye, Isle of Skye, Highland Region, Scotland	
MIR 106	5/2/95	River Schnizort, Dunvegen, Isle of Skye, Highland Region, Scotland	
MIR 107	5/2/95	River at Bernisdale, Isle of Skye, Highland Region, Scotland	
MIR 108	5/2/95	River Drynock, Carbost, Isle of Skye, Highland Region, Scotland	
MIR 109	5/3/95	River at Pentland Road, Isle of Lewis, Highland Region, Scotland	
MIR 110	5/3/95	River Greeta, Pentland, Isle of Lewis, Highland Region, Scotland	
MIR 111	5/3/95	River at Chanais, Boderer, Isle of Lewis, Highland Region, Scotland	
MIR 112	5/3/95	River at Leiniscal, Isle of Lewis, Highland Region, Scotland	
MIR 113	5/3/95	River to Loch Lathainuel, Isle of Lewis, Highland Region, Scotland	
MIR 114	5/4/95	River at Bahii Allen, Isle of Lewis, Highland Region, Scotland	
MIR 115	5/4/95	River at Loch mouth, Tarbet, Isle of Lewis, Highland Region, Scotland	
MIR 116	5/4/95	River at Tarbet, Isle of Lewis, Highland Region, Scotland	
MIR 117	5/5/95	River at Strathkaiard, Ullapool, Highland Region, Scotland	
MIR 118	5/5/95	Loch at Knockau, Ullapool, Highland Region, Scotland	
MIR 119	5/5/95	River at Benmore, Ledmore Junction, Highland Region, Scotland	
MIR 120	5/5/95	River Oakley, Ledmore, Highland Region Scotland	
	-	(Sheet 4 of 7)	

Table A1 (Continued)			
Code	Date	Site	
MIR:121	5/23/95	Cleethorpes Country Park Lake, Cleethorpes, Humberside, England	
MIR 122	5/23/95	Louth Canal, Tetney Lock, south of Cleethorpes, Lincolnshire, England	
MIR 123	5/23/95	Trout Pond (1), Maltby le Marsh, Mabelthorpe, Lincolnshire, England	
MIR 124	5/23/95	River at Yarburgh, Louth, Lincolnshire, England	
MIR 125	5/23/95	Fishing Pond (1), Maltby le Marsh, Mabelthorpe, Lincolnshire, England	
MIR 126	5/24/95	River Bain, Coningsby, Sleaford, Lincolnshire, England	
MIR 127	5/23/95	Fishing Pond (2), Maltby le Marsh, Mabelthorpe, Lincolnshire, England	
MIR 128	5/22/95	Lakes at Ealand, Humberside, England	
MIR 129	5/23/95	Trout Pond (2), Maltby le Marsh, Mabelthorpe, Lincolnshire, England	
MIR 130	6/6/95	Rookley Lake, Rookley Country Park, Isle of Wight, England	
MIR 131	6/7/95	Alvington Manor Pool, Carisbrooke, Isle of Wight, England	
MIR 132	6/12/95	Bala Lake, Gwynedd, Wales	
MIR 133	6/13/95	River Teme, Hereford and Worcestershire, England	
MIR 134-135	6/13/95	River at Oversley Green, Alcester, Hereford and Worcestershire, England	
MIR 136	7/4/95	Lago di Maggiore, Bareno, Arona, Peidmont, Italy	
MIR 137	7/4/95	Lago di Monate, Monate, Lombardi, Italy	
MIR 138	7/4/95	Lago di Varese, Biandronno, Lombardi, Italy	
MIR 139	7/4/95	Lago di Como, Cernobbio, Lombardi, Italy	
MIR 140	7/5/95	Lago di Endine, Sponone al Lago, Lombardi, Italy	
MIR 141	7/5/95	Lago d'Idro, opposite Idro, Lombardi, Italy	
MIR 142	7/6/95	Lago di Garda, Maderno, Lombardi, Italy	
MIR 143	7/6/95	River Site, Quarto d'Altino, Veneto, Italy	
MIR 144	7/6/95	River at Oderzo, Veneto, Italy	
MIR 145	7/6/95	River at Blessaglia, Veneto, Italy	
MIR 146	7/6/95	River at Pordenone->Udine Road, Veneto, Italy	
MIR 147	7/7/95	Lake Bohinjskajez, Ribcev Laz, Slovenia	
MIR 148	7/7/95	Lake Bled, Bled, Slovenia	
MIR 149	7/8/95	Afrilzer See, north of Villach, Austria	
		(Sheet 5 of 7)	

Table A1 (Continued)			
Code	Date	Site	
MIR 150	7/8/95	Brennsee, north of Villach, Austria	
MIR 151	7/8/95	Millstater See, Spittal, Austria	
MIR 152	7/8/95	Mondsee, east of Salzburg, Austria	
MIR 153	7/8/95	Attersee, east of Salzburg, Austria	
MIR 154	8/7/95	Lochgelly, northeast of Dumferlin, Fife, Scotland	
MIR 155	8/7/95	Loch Ore, Ballingry, Fife, Scotland	
MIR 156	8/7/95	River Tay, Perth Racecourse, Tayside, Scotland	
MIR 157	8/8/95	River South Esk, Brechin->Forfar, Tayside, Scotland	
MIR 158	8/8/95	River Don, Inverurie, Grampian Region, Scotland	
MIR 159	8/8/95	River Ythan, Methlick, Grampian Region, Scotland	
MIR 160	8/8/95	River Deveron, Turriff, Grampian Region, Scotland	
MIR 161	8/9/95	Loch Morlich, east of Aviemore, Highland Region, Scotland	
MIR 162	8/9/95	Loch an Eilein, south of Aviemore, Highland Region, Scotland	
MIR 163	8/9/95	Loch Insh, south west of Aviemore, Highland Region, Scotland	
MIR 164	8/9/95	Loch Tay, Kenmore Tayside, Scotland	
MIR 165	8/9/95	Loch on River Dochen, Benmore, Central Region, Scotland	
MIR 166	8/10/95	Loch Lomond, Inveriglas, Strathclyde, Scotland	
MIR 167	8/10/95	Loch above Loch Long, Strathclyde, Scotland	
MIR 168	8/10/95	Crinan Canal, Lochgilphead, Kintyre, Strathclyde, Scotland	
MIR 169	8/10/95	River Add, Bridgend, Kintyre, Strathyclyde, Scotland	
MIR 170	8/10/95	Loch Coille-Bharr, Knapdale Forest, Kintyre, Strathclyde, Scotland	
MIR 171	8/10/95	Loch Eck, north of Donoor, Strathclyde, Scotland	
MIR 172	8/11/95	Loch Ascog, Isle of Bute, Strathclyde, Scotland	
MIR 173	8/11/95	River Leven, Renton, north of Dumbarton, Strathclyde, Scotland	
MIR 174	8/11/95	Carman Reservoir, Renton, north of Dumbarton, Strathclyde, Scotland	
MIR 175	8/9/95	Loch on B846, below Rannoch, Tayside, Scotland	
MIR 176	9/21/95	Embalsa del Ebro, Canlabrica, Spain	
MIR 177	9/22/95	Embalsa de Aguilar de Campo, Aguilar, Spain	
MIR 178	9/22/95	Rio Rivero, Ruesaga, Spain	
		(Sheet 6 of 7)	

Table A1 (Concluded)		
Code	Date	Site
MIR-179	9/22/95	Rio Carrion, Velilla delCarrion, Spain
MIR 180	9/23/95	Rio Sil, Ponferrada, between Villa Patos and Toraldelosv, Spain
MIR 181	9/23/95	Rio Sil, Ponferrada, below Penarrubia dam and Salas de la Ribera, Spain
MIR 182	9/24/95	Lago de Sanabria, above Puebla Sanabria, Spain
MIR 183	9/24/95	Rio Tera, Puebla Sanabria, Spain
MIR 184	9/24/95	Rio Sabor, south of Rabal, Portugal
MIR 185	9/24/95	Rio Igrejas, Gamonde, Portugal
MIR 187	9/24/95	Rio Macas, Spanish Portuguese border, Portugal
MIR 188	9/25/95	Rio Coa, Vilar to Sabugal Road, Portugal
MIR 189	9/25/95	Rio Zezere, Caria to Teixosa Road, Portugal
MIR 190	9/25/95	Rio Dao, N231 north of Constancia, Portugal
MIR 191	9/26/95	Rio Tejo, south of Constancia, Portugal
MIR 192	10/18/95	Chester Canal, Chester, Cheshire, England
MIR 193	10/17/95	Llyn, Clwyd, Wales
-		(Sheet 7 of 7)

# Appendix B Fungal Species Isolated From Myriophyllum spicatum During 2 Years of Surveying in Europe

Absidia cylindrospora Hagem.

Acremonium strictum W. Gams.

Acremonium persicinum (Nicot.) W. Gams.

Acrophialophora levis Samson and T. Mahmood.

Alternaria infectoria E. G. Simmons. Agg.

Apiospora montagnei Sacc.

Ascochyta sp. Lib.

Aureobasidium sp. Viola and Boyer.

Byssochlamys nivea Westling.

Botrytis cinerea Pers.

Chrysosporium sp. Corda

Cladobotryum sp. Corda

Colletotrichum dematium (Pers.:Fr.) Grove.

Coniothyrium fuckelii Sacc.

Coniothyrium sporulosum (W. Gams and Domsch) Aa.

Corynascus sepedonium (Emm.) Arx.

Cryptosporiopsis sp. Bub. and Kabat.

Cylindrocarpon destructans (Zinssm.) Scholten.

Cylindrocarpon aquaticum (Sv. Nilsson) Maranova and Descals

Cylindrocarpon sp. Morgan

Embellisia sp. Embellisia cf. telluster E. G. Simmons.

Emericellopsis minima Stolk.

Fusarium acuminatum Ellis and Everhart

Fusarium avenaceum (Fr.) Sacc.

Fusarium crookwellense Burgess, P. E. Nelson and Touss.

Fusarium culmorum (W.G.Sm.) Sacc.

Fusarium equisiti (Corda) Sacc.

Fusarium flocciferum Corda.

Fusarium graminearum Schwabe.

Fusarium oxysporum Schlecht.

Fusarium poae (Peck) Wollenweber.

Fusarium sambucinum Fuckel

Fusarium solani (Martius) Sacc.

Fusarium sporotrichioides Sherb.

Fusarium sp. Link.

Geotrichum candidium Link.

Gliocladium catenulatum J. C. Gilman and E. V. Abbott.

Gliocladium roseum Banier.

Gliomastix murorum var. felina (Marchal) S. Hughes.

Glomerella cingulata (Stoneman) Spauld. and H. Schrenk.

Microdochium tabacinum (T. H. Beyma) Arx.

Microsphaeropsis sp. Höhn

Mycocentrospora acerina (Hartig) Deighton.

Myrothecium cinctum (Corda) Sacc.

Myrothecium roridum Tode.

Nectria discophora (Mont.) Mont.

Nectria lugdunensis J. Webster

Phaeoseptoria sp. Speg.

Phoma complanata (Tode) Desm.

Phoma dennisii Boerema.

Phoma eupyrena Sacc.

Phoma exigua Desm.

Phoma hedericola (Dur. and Mont.) Boerema.

Phoma leveillei Boer. and G. J. Bollen.

Phoma macrostroma Mont.

Phoma nebulosa (Pers.:Fr.) Berk.

Phoma tropica R. Schneid. and Boerema.

Phoma sect. Paraphoma (Morgan-Jones and White) Boerema

Phoma sp. Desm.

Phomopsis sp. Sacc.

Pithomyces chartarum (Berk. and M. A. Curtis) M. B. Ellis

Plectospaerella cucumerina (Lindf.) Gams.

Pythium sp. Pringsh.

Pythium sp. group F

Pythium sp. group HS

Pythium sp. group T

Pythium aquatile Höhnk.

Pythium acanthophoron Sideris.

Pythium periplocum Drechsler.

Pythium scleroteichum Drechsler.

Sclerotium hydrophilum Sacc.

Stagonospora sp. Sacc.

Saprolegnia parasitica Coker.

Trichosporiella sporotrichoides Oorschot.

Verticillium nigrescens Pethybr.

# Appendix C Isolates That Have Been Screened Against Sections of Myriophyllum spicatum

Code	Isolate	Result
MIR 1	Plectosphaerella cucumerina	No response
MIR 2	Fusarium sp.	No response
MIR 2y	Acremonium strictum	No response
MIR 2iii	Gliocladium roseum	No response
MIR 2ia	Pythium sp.	No response
MIR 3iii	Embellisia nr. telluster	Good control
MIR 3a	Embellisia nr. telluster (reisolated 3iii)	Good control
MIR 4vi	Acremonium strictum	No response
MIR 4xa	Fusarium sporotrichoides	No response
MIR 5v	Fusarium crookwellense	No response
MIR 5i	Apiospora montagnei	No response
MIR 5ix	Acremonium strictum	No response
MIR 5e	Byssochylamys nivea	No response
MIR 5va	Fusarium crookwellense	No response
MIR 5iv	Fusarium sporotrichoides	No response
MIR 5xv	Fusarium sporotrichoides	No response
MIR 5iii	Acremonium persicinum	No response
MIR 6	Verticillium nigrescens	No response
MIR 6vi	Acremonium strictum	No response

Code	Isolate	Result
MIR 7a	Aureobasidium sp.	No response
MIR 7xii	Acremonium strictum	No response
MIR 7xiii	Fusarium avenaceum	No response
MIR 8b	Acremonium sp.	No response
MIR 13ii	Fusarium sambucinum	No response
MIR 13i	Fusarium sambucinum	No response
MIR 16ii	Fusarium graminearum	No response
MIR 16ii	Fusarium sambucinum	No response
MIR 16i	Fusarium avanaceum	No response
MIR 16iii	Fusarium culmorum	No response
MIR 16vii	Fusarium culmorum	No response
MIR 16	Fusarium solani	Good control
MIR 16b	Fusarium oxysporum	No response
MIR 16a	Fusarium acuminatum	No response
MIR 17j	Acremonium strictum	No response
MIR 17	Fusarium graminearum	No response
MIR 18	Mucor hiemalis	No response
MIR 18	Alternaria alternata	No response
MIR 18c	Coniothyrium sporulosum	No response
MIR 18a	Coniothyrium sporulosum	No response
MIR 22i	<i>Verticillium</i> sp.	No response
MIR 22	Fusarium polyphialides	No response
MIR 22r	Fusarium oxysporum	No response
MIR 23	Embellisia indefessa	No response
MIR 23	Ascochyta sp.	No response
MIR 23	Fusarium crookwellense	No response
MIR 24	Cylindrocladium sp.	No response
MIR 24i	Mucor hiemalis	No response
MIR 25	Cylindrocladium sp.	No response
MIR 25ii	Gliocladium roseum	No response
MIR 25iii	Gliocladium roseum	No response
MIR 25i	Gliocladium roseum	No response
		(Sheet 2 of 10

Table C1 (Continued)		
Code	Isolate	Result
MIR 25iv	Gliocladium roseum	No response
MIR 26	Oomycete	No response
MiR 26a	Fusarium graminearum	No response
MIR 26	Acremonium sp.	No response
MIR 27e	Gliocladium roseum	No response
MIR 27d	Gliocladium roseum	No response
MIR 27f	Acremonium sp.	No response
MIR 27ii	Trichosporiella sporotrichoides	No response
MIR 27g	Pythium aquatile	No response
MIR 27i	Epicoccum nigrum	No response
MIR 28	Pythium scleroteichium	No response
MIR 29b	Acremonium sp.	No response
MIR 29c	Cylindrocarpon sp.	No response
MIR 30	Phomopsis sp.	No response
MIR 30b	Cylindrocarpon sp.	No response
MIR 30a	Cylindrocladium sp.	No response
MIR 30	Phoma sp.	No response
MIR 30i	Stagonospora sp.	No response
MIR 30	Pythium sp.	No response
MIR 31a	Fusarium oxysporum	No response
MIR 32	Phoma sp.	No response
MIR 32d	Indeterminate Hyphomycete	No response
MIR 32	Acremonium sp.	No response
MIR 32a	Cylindrocarpon sp.	No response
MIR 32b	<i>Verticillium</i> sp.	No response
MIR 32c	Fusarium culmorum	No response
MIR 34	Gliocladium roseum	Good control
MIR 34a	Fusarium sp.	No response
MIR 34b	Corynascus sepedonium	No response
MIR 35	Fusarium sambucinum	No response
MIR 35	Fusarium graminearum	No response
MIR 35	Indeterminate Coelomycete	Good control
(Sheet 3 of 10)		

Table C1 (Continued)		
Code	Isolate	Result
MIR 35a	Oomycete	No response
MIR 36e	Indeterminate Ascomycete	No response
MIR 36	Gliocladium roseum	No response
MIR 36b	Mortierella sp.	No response
MIR 36	Gliomastix murorum var. felina	No response
MIR 36ii	Indeterminate Hyphomycete	No response
MIR 37b	Phomopsis sp.	No response
MIR 37d	Acremonium sp.	No response
MIR 38b	Phomopsis sp.	No response
MIR 38	Acremonium sp.	No response
MIR 38a	Absidia cylindrospora	No response
MIR 40a	Acremonium sp.	No response
MIR 42	Cylindrocladium sp.	No response
MIR 42b	Fusarium sambucinum	No response
MIR 42	Fusarium sambucinum	No response
MIR 42	Cladobotryum sp.	No response
MIR 43a	Fusarium sambucinum	No response
MIR 43	Fusarium sambucinum	No response
MIR 43c	Fusarium pallidoroseum	No response
MIR 43e	Emericellopsis minima	No response
MIR 43f	Acremonium sp.	No response
MIR 43	Acremonium sp.	No response
MIR 43	Phoma exigua	No response
MIR 43	Phomopsis sp.	No response
MIR 43	Fusarium sambucinum	No response
MIR 44	Indeterminate Hyphomycete	No response
MIR 44	Saprolegnia parasitica	No response
MIR 44a	Cylindrocladium sp.	No response
MIR 44	Oomycete	No response
MIR 45e	Oomycete .	No response
MIR 45f	Cylindrocarpon destructans	No response
MIR 45a	Cylindrocarpon destructans	No response
		(Sheet 4 of 10)

Table C1 (Continued)		
Code	Isolate	Result
MIR 45c	Cylindrocarpon destructans	No response
MIR 45b	Acremonium sp.	No response
MIR 45	Acremonium sp.	No response
MIR 45d	Oomycete	No response
MIR 45h	Indeterminate Hyphomycete	No response
MIR 47a	Cladobotryum sp.	No response
MIR 48	Oomycete	No response
MIR 49	Cylindrocarpon sp.	No response
MIR 49a	Indeterminate Hyphomycete (reisolated MIR 49d)	Good control
MIR 49b	Gliocladium roseum	No response
MIR 49g	Acremonium sp.	No response
MIR 49d	Indeterminate Hyphomycete	Good control
MIR 50	Acremonium sp.	No response
MIR 50	Acremonium sp.	No response
MIR 51	Glomerella cingulata	Good control
MIR 58	Oomycete	No response
MIR 59	Chrysosporium sp.	No response
MIR 59d	Geotrichum candidum (reisolated MIR 59e)	No response
MIR 59e	Geotrichum candidum	Not retested
MIR 59	Geotrichum candidum (reisolated 59c)	Good control
MIR 59c	Geotrichum candidum	Not retested
MIR 59g	Chrysosporium sp.	No response
MIR 59	Indeterminate Hýphomycete	No response
MIR 60a	Fusarium equiseti	No response
MIR 64b	Cylindrocladium sp.	No response
MIR 64c	Coniothyrium fuckelii (reisolated 64d)	Good control
MIR 64d	Coniothyrium fuckelii	Not retested
MIR 65a	Gliocladium roseum (reisolated 65b)	Slight effect
MIR 65b	Gliocladium roseum	Not retested
MIR 67a	Pythium periplocum	Slight effect
MIR 67c	Verticillium sp.	No response
MIR 68a	Fusarium sambucinum	No response
		(Sheet 5 of 10)

Table C1 (Continued)		
Code	Isolate	Result
MIR 68g	Gliocladium roseum	No response
MIR 68h	Gliocladium roseum	No response
MIR 68c	Acremonium sp.	Good control
MIR 68a	Fusarium sambucinum	No response
MIR 69a	Indeterminate Hyphomycete	No response
MIR 70c	Acremonium sp.	No response
MIR 70a	Acremonium sp.	No response
MIR 71a	Phomopsis sp.	No response
MIR 73c	Fusarium avenaceum	No response
MIR 75b	Indeterminate Hyphomycete	No response
MIR 75a	Cylindrocladium sp.	No response
MIR 76a	Acremonium sp.	No response
MIR 77a	Fusarium coeruleum	No response
MIR 78b	Cylindrocladium sp. (reisolated 78g)	No response
MIR 78g	Cylindrocladium sp.	No response
MIR 78a	Fusarium ciliatum	No response
MIR 79a	Fusarium sambucinum	No response
MIR 80a	Fusarium graminearum	No response
MIR 80b	Cylindrocarpon destructans	Good control
MIR 80c	Indeterminate Hyphomycete	Good control
MIR 80j1	Indeterminate Coelomycete	No response
MIR 83a	Acremonium sp.	No response
MIR 84a	Mycocentrospora acerina	No response
MIR 85a	Acremonium sp.	No response
MIR 86b	Fusarium sp.	No response
MIR 86a	Lemonniera sp.	No response
MIR 87c	Leptosphaerulina sp.	No response
MIR 87b	Fusarium sporotrichoides	No response
MIR 89b	Acremonium sp.	No response
MIR 89e	Fusarium sp.	No response
MIR 91b	Gliocladium sp.	No response
MIR 92a	Fusarium avenaceum	No response
(Sheet 6 of 10)		

Table C1 (Continued)		
Code	Isolate	Result
MIR-93c	Gliocladium roseum	No response
MIR 93b	Gliocladium roseum (reisolated 93g)	Good control
MIR 93g	Gliocladium roseum	Not retested
MIR 93e	Acremonium sp.	No response
MIR 94d	Indeterminate Coelomycete	No response
MIR 96b	Fusarium sp.	No response
MIR 97c	Colletotrichum sp.	No response
MIR 100a	Fusarium culmorum	No response
MIR 101a	Fusarium culmorum	No response
MIR 102a	Fusarium sp.	No response
MIR 102c	Fusarium equiseti	No response
MIR 102j2	Cylindrocladium sp.	No response
MIR 103c	New Hyphomycete	No response
MIR 104a	Macrophoma sp.	No response
MIR 108a	Phaeoseptoria sp.	No response
MIR 113a	Coniothyrium sp.	No response
MIR 114a	Phoma sp.	No response
MIR 115b	Phaeoseptoria sp.	No response
MIR 115a	Ascochyta sp.	No response
MIR 116a	Coniothyrium sp.	No response
MIR 117a	Coniothyrium sp.	No response
MIR 119a	Indeterminate Hyphomycete	No response
MIR 119j1	Fusarium acuminatum	No response
MIR 120	Indeterminate Hyphomycete	No response
MIR 122a	Acremonium sp.	No response
MIR 123b	Acremonium sp.	No response
MIR 124b	Indeterminate Hyphomycete	No response
MIR 124a	Phoma sp.	No response
MIR 125b	Acremonium sp.	No response
MIR 125a	Cladosporium cladosporioides	No response
MIR 126a	Phomopsis sp.	No response
MIR 126b	Phoma sp.	No response
(Sheet 7 of 10)		

Table C1 (Continued)		
Code	Isolate	Result
MIR 126c	Acremonium sp.	No response
MIR 126d	Fusarium equiseti	No response
MIR 127b	Acremonium sp.	No response
MIR 128a	Acremonium sp.	No response
MIR 128b	Fusarium sp.	No response
MIR 129c	Fusarium sp.	No response
MIR 129d	Fusarium sp.	No response
MIR 129j1	Sclerotial isolate	No response
MIR 131b	Fusarium sp.	No response
MIR 131a	Oomycete	No response
MIR 132a	Pythium acanthophoron	No response
MIR 133a	Acrophialophora levis	No response
MIR 134a	Cryptosporiopsis sp.	Good control
MIR 134c	Coniothyrium sp.	No response
MIR 135j1	Coniothryium sp.	No response
MIR 136a	Phoma sect. Paraphoma	No response
MIR 138b	Indeterminate Hyphomycete	No response
MIR 139c	Mucor sp.	No response
MIR 139a	Alternaria sp.	No response
MIR 139b	Myrothecium roridum	No response
MIR 140b	Fusarium sambucinum	No response
MIR 140a	Phoma sp.	No response
MIR 140j1	Gliocladium sp.	No response
MIR 141c	Fusarium sp.	No response
MIR 141a	Phoma sp.	No response
MIR 142a	Pithomyces chartarum	No response
MIR 142b	Acremonium sp.	No response
MIR 143c	Fusarium sp.	No response
MIR 143a	Fusarium sp.	No response
MIR 144b	Indeterminate Hyphomycete	No response
MIR 144b	Acremonium sp.	No response
MIR 144j1	Alternaria sp.	No response
(Sheet 8 of 10)		

Table C1 (Continued)		
Code	Isolate	Result
MIR 144j2	: Fusarium culmorum	No response
MIR 145c	Fusarium sp.	No response
MIR 145a	Coniothyrium sp.	No response
MIR 147a	Alternaria sp.	No response
MIR 147c	Myrothecium sp.	No response
MIR 148a	Indeterminate Coelomycete	No response
MIR 148b	Ascochyta sp.	No response
MIR 148c	Fusarium sp.	No response
MIR 149a	Sclerotium hydrophilum	No response
MIR 149b	Sclerotium hydrophilum	No response
MIR 150a	Ascochyta sp.	No response
MIR 151a	Ascochyta sp.	No response
MIR 152a	Fusarium sp.	No response
MIR 156a	Alternaria sp.	No response
MIR 157a	Fusarium graminearum	No response
MIR 158e	Acremonium sp.	No response
MIR 158a	Ascochyta sp.	No response
MIR 158b	Ascochyta sp.	No response
MIR 158c	Ascochyta sp.	No response
MIR 158d	Ascochyta sp.	No response
MIR 159c	Cylindrocladium sp.	No response
MIR 159a	Cylindrocladium sp.	No response
MIR 159b	Phoma sp.	No response
MIR 160a	Acremonium sp.	No response
MIR 161a	Indeterminate Hyphomycete	No response
MIR 151b	Alternaria sp.	No response
MIR 162a	Oomycete	No response
MIR 163a	Oomycete	No response
MIR 163b	Oomycete	No response
MIR 163c	Indeterminate Hyphomycete	No response
MIR 164j1	Ascochyta sp.	No response
MIR 164j2	Phaeostalagmus sp.	No response
(Sheet 9 of 10)		

Table C1 (Concluded)		
Code	Isolate	Result
MIR 164a	Indeterminate Hyphomycete	No response
MIR 166j2	Phoma tropica	No response
MIR 166j3	Phoma sect. Paraphoma	No response
MIR 166j1	Phoma dennisii	No response
MIR 167j2	Phoma sp.	No response
MIR 168j2	Nectria lugdunensis	No response
MIR 168j1	Cylindrocarpon aquaticum	No response
MIR 169j1	Indeterminate Coelomycete	No response
MIR 169j2	Indeterminate Coelomycete	No response
MIR 169j3	Indeterminate Coelomycete	No response
MIR 170j1	Indeterminate Coelomycete	No response
MIR 171j1	Phoma leveillei	No response
MIR 172j1	Phoma hedericola	No response
MIR 173j1	Phomopsis sp.	No response
MIR 174j1	Coniothrium sp.	No response
, , , ,		(Sheet 10 of 10)

#### REPORT DOCUMENTATION PAGE

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surveyed for fungal pathogens 400 potential pathogens in 38 on sections of plants; of these,	that could be used as bioco- genera were obtained in pur 13 have been shown to po wo indeterminate Hyphom imbellisia nr. telluster, Fus	ontrol agents against Myrio are culture. Isolates have b assess some control capabil aycetes (producing only chlar arium solani, Geotrichum	neen screened for pathogenicity ities. These include two iso-amydospores), Acremonium sp., candidum, Coniothyrium
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